Identifying therapeutic targets by elucidating signaling pathways in pediatric lymphoid leukemias
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Brief communication: Targets for the treatment of lymphoid blast crisis Chronic Myeloid Leukemia?

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ABSTRACT

Once chronic myeloid leukemia has progressed to blast crisis (BC), median overall survival rates are limited to only a few months, despite the use of second and third generation tyrosine kinase inhibitors (TKIs).\textsuperscript{1-3} Even when TKIs are combined with intensive chemotherapy protocols, as used for the treatment of pediatric acute leukemias, no improvements in outcome are observed.\textsuperscript{3,4} Therefore, new treatment options are urgently needed.

How CML chronic phase transforms into blast phase is not completely understood; making it difficult to design and implement new treatment strategies. Currently, it is hypothesized that blast crisis is the direct result of continued BCR-ABL activity; causing DNA damage and impaired DNA repair through oxidative stress and reactive oxygen species.\textsuperscript{3,5-7} This ultimately result in the accumulation of various genetic abnormalities besides the Philadelphia chromosome as well as the activation of other signaling pathways, independent of BCR-ABL1 activity.\textsuperscript{2,3,7} However, which signal transduction pathways or specific signaling hubs are activated in blast crisis CML is largely unknown, especially in lymphoid blast crisis CML (CML-LyBC).

Previous studies have described a roll for Akt, STAT3, and FAK activation in the development of imatinib resistance in CML, with a subsequent higher risk of progression to blast crisis.\textsuperscript{8-13} We hypothesize that BCR-ABL1 independent activation of these targets might be involved in CML-LyBC cell survival and therefore, inhibition of these targets might be of potential interest as new targets for the treatment of lymphoid blast crisis CML. Hence we have studied the role of Akt, STAT3, and FAK for the survival of CML-LyBC cells to identify new potential therapeutic targets.

Patients’ characteristics are shown in Supplementary Table 1. Western blot analysis was performed to study phosphorylation levels of Akt, STAT3, and FAK, as well as effects of imatinib treatment on the activation of these targets. Subsequently, the effect of pharmacological inhibition of these targets on cell survival was determined using Annexin V / PI staining. As expected, cell viability was not or only slightly influenced by imatinib (Figure 1).

Western blot analyses showed Akt\_S473 and FAK\_Y397 expression in two CML-LyBC cases (cases #1 and #2), as well as a prominent phosphorylation of STAT3\_Y705 in CML-LyBC#2 (Figure 2A). No phosphorylation of Akt\_S473, STAT3\_Y705, and FAK\_Y397 was observed in CML-LyBC#4 (Figure 2A). Additionally, autophosphorylation of these targets was unaffected by imatinib treatment (Figure 2A). In accordance with our Western blot results, CML-LyBC cases #1 and #2 were sensitive to the combination of imatinib with the Akt inhibitor MK2206, while LyBC#2 was also highly sensitive to combinational treatment with the STAT3 inhibitor S3I-201 (Figure 2B). As FAK phosphorylation levels were low in all samples, cell viability was only slightly influenced by the combination of PF-431396 (FAK inhibitor) and imatinib (Figure 2B). As expected, no responses upon combination treatment were observed in LyBC#4 (Figure 2B).
Figure 1 Effects of imatinib treatment on cell viability

Effects of imatinib treatment on cell viability after 48 hours normalized to DMSO treated control cells. Cell viability was determined using Annexin V-FITC/PI staining. The CML-LyBC cell line BV-173 was used as a positive control. Results are shown as mean ± standard deviation. Asterisks (*) indicate significant differences ($P \leq 0.05$) by paired two-tailed $t$-test.

In conclusion, phosphorylation of three targets, implicated in imatinib resistance e.g. Akt, STAT3, and FAK, was highly variable between four CML-LyBC cases, and consistently, differential effects of target inhibition on cell survival were seen. Nevertheless, these preliminary results show that the direct use of kinase inhibitors might be suitable for the treatment of CML-LyBC, guided by kinase activity profiles in individual patients. This further emphasizes the need for personalized approaches to identify patient-specific targets for therapy. Hence, to extend on these preliminary observations, kinome and proteome profiling may be successfully used to identify active signal transduction pathways in CML-LyBC.
Figure 2 Akt, STAT3, and FAK expression and inhibition in primary CML-LyBC

(A) Western blot results of Akt_S473, STAT3_Y705, and FAK_Y397 in four CML-LyBC cases after treatment with 1 μM imatinib for 24 hours.

(B) Cell survival in response to combinational treatment of imatinib (1 μM) and MK2206 (1 μM, Akt inhibitor), S3I-201 (10 μM, STAT3 inhibitor), or PF-431396 (1 μM, FAK inhibitor) for 48 hours normalized to DMSO treated control cells. Cell viability was determined using Annexin V-FITC/PI staining. Results are shown as mean ± standard deviation. Asterisks (*) indicate significant differences (P ≤ 0.05) by paired two-tailed t-test.
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SUPPLEMENTARY INFORMATION

Supplementary table 1 Patients’ characteristics

<table>
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<tr>
<th>Patient ID</th>
<th>Age (years)</th>
<th>Year of diagnosis BC</th>
<th>Prior treatment with TKI</th>
<th>Death (months after BC diagnosis)</th>
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<tr>
<td>CML-LyBC#1</td>
<td>14.9</td>
<td>1999</td>
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<tr>
<td>CML-LyBC#2</td>
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<tr>
<td>CML-LyBC#3</td>
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<td>Unknown</td>
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<td>CML-LyBC#4</td>
<td>11.3</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Yes (7.5)</td>
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</tbody>
</table>

Abbreviations: BC = Blast Crisis
REFERENCES


